

**GENE EDITING FOR CANADIAN FIELDS CROPS – TARGETS AND
BENEFITS**

MARCH 31ST 2021

Contents

Executive Summary	1
1. Fixing Desirable Single Gene Traits in Breeding Programs	2
2. Gene Editing to Promote Domestication.....	3
3. CRISPR/Cas9 for Generating Novel and Superior Alleles.....	4
4. Modulating Gene Recombination in Plants Using Gene Editing	6
5. Durable Crop Disease Resistance.....	7
6. Reducing Gluten Immunogenicity AND IMPROVING GRAIN QUALITY in Wheat	9
7. Gene Editing for Improved Quality of Barley.....	10
8. INCREASED Nutritional Value of Canola Oil and Seeds, using CRISPR/Cas9.....	11
9. CRISPR/Cas9 for Improved Soybean Oil Quality and Early Flowering	12
10. Improving Fiber Quality and Seed Oil Composition in Flax using Gene Editing	13
11. Gene Editing could improve Maize Salt tolerance and Lodging Resistance.....	14
12. Tomato with Improved Bioactive Components.....	15
13. Preserving Organoleptic Properties in Potato and Eggplant	16
14. Vicine-free Faba bean Cultivars	17
15. Eliminating the off-flavour of pea protein.....	18
Summary	19
References	21

This paper was compiled by Amidou N’Diaye (lead author) and Curtis Pozniak (supervising author), University of Saskatchewan.

Executive Summary

With the fast-growing global population, which is projected to reach 9.7 billion people in 2050, there will be an unprecedented demand for food crops, animal feed and biofuel. However, global crop yields are plateauing as a result of unproductive cyclical weather patterns, and increased spread of diseases and pests. The vanishing of biodiversity of ecosystems and diversity of agricultural crops along with loss of arable land has increased the demand for more sustainable and precise agricultural practices. A major challenge for plant breeders is developing environmentally resilient cultivars that perform in a stable manner in response to changing agronomic practices and variable environments conditions resulting from climate change. Plant breeders rely on genetic variability, breeding tools, and methods to rearrange desirable genes into commercial cultivars – largely focusing on traits such as higher yield, disease resistance, abiotic stress (heat, drought, cold) tolerance, and improvement of nutritional quality of foods for a growing world population. Pathogens damage crops and rob yield potential, and an important component of plant breeding is to stay ahead of this curve, by identifying and incorporating durable disease resistance to protect the crop without the need for chemical control of pathogens.

Crop breeding has benefited from modern technologies to support cultivar development. This is particularly true in case of some of the world's most important crops – for example, maize, wheat and rice. Molecular markers have increased breeding efficiency by selection of traits based on genomic information rather than by phenotyping. However, despite the benefit of molecular tools and genomic information, combining multiple desirable agronomic outcomes is still hindered by genetic correlations between traits. The rapid development of CRISPR (clustered regularly interspaced short palindromic repeats)/Cas (CRISPR associated proteins)-based gene editing has created an alternative avenue for crop improvement and has the potential to increase speed and precision in plant breeding programs. Due to its high precision, ease of design, multiplexing ability, and low cost, this innovative technology has the potential to revolutionize agriculture. Here, we summarize examples of the integration of CRISPR/Cas-based gene editing into to create varieties for various applications and growth environments. We

highlight the use of CRISPR/Cas-based gene editing to accelerate *de novo* domestication, generate novel/superior alleles in elite lines, and “promoter bashing” for increased resilience of crops to abiotic stresses. While our examples have been abbreviated, a more detailed review has been published recently (Lyzenga et al. 2021). Furthermore, we summarize how CRISPR/Cas9 gene editing is being used to engineer durable disease resistance to various pathogens and pests, to increase quality and nutritional traits of new crop varieties and to eliminate ‘anti-nutritional’ compounds in crop plants. While survey results reveal a consensus on the potential of gene-editing on crop improvement, health and safety regulations, followed by export markets, consumers, and the media play a major role in determining its adoption on a larger scale.

1. Fixing Desirable Single Gene Traits in Breeding Programs

Some plant traits are under simple genetic control, usually being regulated by only one or a few genes. In some cases, these genes have been sequenced and their functional influence on trait expression has been confirmed. For example, in durum wheat, the *Cdu-B1* gene controls cadmium (Cd) accumulation in durum wheat grain, and the low Cd allele codes for a heavy metal transporter that locks the Cd in root cells, preventing the harmful heavy metal from accumulating in the grain. High accumulation results from a small DNA insertion into the gene, rendering it ineffective in sequestering Cd into roots. In this case, Cd freely moves and translocates to the grain. Gene editing can be used to precisely convert this undesirable allele of *Cdu-B1* into favorable low Cd allele in germplasm. Knowing the function of this heavy metal transporter provides an opportunity where gene editing technology could be used to edit similar genes in other crops that accumulate Cd, such as in Flax. Gene editing could be coupled with rapid-generation cycling to quickly produce productive lines with desirable alleles.

There are several other examples of monogenic improvement traits which have functionally characterized genes/alleles. For example, the wheat dwarfing alleles Rht-B1b (formerly Rht1) and Rht-D1b (Rht2) each contain a single base-pair mutation which gives rise to a premature STOP codon, resulting in a truncated protein with altered function in gibberellin signal transduction. The dwarfing alleles confer reduced plant height, resulting in increased harvest index. Similarly, the null allele of grain weight 2 (GW2), a regulator of cell division, leads to an increase in grain

width and weight in rice and wheat (Song et al., 2007; Simmonds et al., 2016; Wang et al., 2018; Zhang et al., 2018). The dwarfing and GW2 alleles could be precisely targeted by gene editing in breeding programs. By fixing a collection of monogenic traits plant breeders could phenotype and perform selections from a population of plants which already have a basic complement of non-segregating traits, such as plant height and grain weight. Plant breeders would thus have more resources to explore and select for other important and more genetically complex traits.

Furthermore, gene editing can be used to save near-miss varieties. Often during the 8-12 year breeding process, new varieties are developed which exhibit many desirable features such as high yield and superior end-use quality/nutrition. However, unanticipated changes, like changes in a pathogen population or disease susceptibility negatively associated with some agronomic trait may impact the viability of the new variety. For example, in wheat resistance to Fusarium head blight (FHB) is highly desirable; however, susceptibility to FHB is associated with the semi-dwarfing stature, perhaps due to linkage. While FHB resistance is multifaceted, and thus more challenging for gene editing experiments, semi-dwarfing allele is simple and thus could be the target of gene editing technology. Gene editing technology provides a faster and precise approach to generate desirable alleles of dwarfing genes into the otherwise agronomically superior cultivars.

2. Gene Editing to Promote Domestication

Domestication followed by intensive breeding have resulted in a genetic bottleneck and many modern crop germplasms have genomic regions of reduced genetic diversity (Shi and Lai, 2015). Trait variation from landraces and wild relatives represent a rich reservoir of genetic variation that can be introduced through introgression breeding. However, this process is tedious and time consuming. Incorporating the beneficial allelic variation into elite lines and leaving behind unadapted genetic material is a major challenge and may not be successful because of introgression across species barriers. However, CRISPR/Cas based gene editing has emerged as tool for generation of novel and superior alleles within crop germplasm or within elite lines (Rodríguez-Leal et al., 2017; Nogue et al., 2016; Shen et al., 2017). In contrast to random mutagenesis through ethyl methanesulphonate (EMS) and gamma irradiation, CRISPR/Cas based

gene editing can be targeted to genomic regions of interest such as promoters, developmental regulators, and transcription factors to promote semi-random mutagenesis. Since CRISPR/Cas based gene editing can be easily multiplexed, multiple genetic regions can be targeted at once.

Recent technological advances have raised the possibility of *de novo* domestication (i.e., the introduction of domestication genes into non-domesticated species) of wild plants as a viable solution to promote the use of wild relatives in plant breeding (Fernie and Yan 2019). Indeed wild relatives of modern and orphan crops were mainly regarded as a source of novel genetic variation particularly for disease resistance. However, traits such as small fruit size, low yield, excessive height and seed dispersal resulting from shattering, constrain their use in breeding programs targeting commercial varieties. Recently, the concept of *de novo* domestication through gene editing has been explored as an exciting possibility to “adapt” wild germplasm by editing genes influencing these undesirable characteristics (Zsögön et al., 2017; 2018). This is plausible using a CRISPR/Cas9 gene editing platform because most domestication genes are well characterized and have simple genetic architecture. There are several examples of how domestication genes were targeted in biotechnology approaches that predate the development and widespread adoption of genome-editing techniques (Østerberg et al. 2017). The use of CRISPR technology has recently been demonstrated in tomato (Li et al. 2018b; Zsögön et al. 2018), where simultaneous editing of six loci important in domestication resulted in increased fruit number (MULT), size (FW2.2, FAS), shape (O gene; OVATE), nutritional content (LYCOPENE BETA CYCLASE) and plant architecture (SP gene; SELF-PRUNING) (Zsögön et al. 2018). Similarly, domestication genes impacting day-length insensitivity (SP5G), fruit size (SICLV3, SIWUS), vitamin C levels (SIGGP1) and plant architecture (SP) were stacked in accessions of *Solanum pimpinellifolium* with disease and salt tolerance (Li et al. 2018b). These studies demonstrate that CRISPR/Cas based gene editing can accelerate domestication and increase the value and use of orphan crops or wild relatives in plant breeding.

3. CRISPR/Cas9 for Generating Novel and Superior Alleles

Most traits targeted by breeders for improvement are controlled by multiple genes, where expression is quantitative, and influenced by the environment. For example, resistance

to cold tolerance in cereal crops is genetically complex, and multiple genes have been implicated in improved cold tolerance. Many studies support that the relative level of gene expression of many interacting genes regulates the cold tolerance pathway – meaning there are multiple genes, each regulated differently, depending on environment. This creates a challenge for gene-editing as it is not always clear “which gene should be edited”, and “what should the precise edit be”. One approach to deal with these complex traits via gene editing as a tool to develop superior alleles within crop germplasm or within elite lines is to use “promoter bashing” (Nogué et al. 2016; Rodríguez-Leal et al. 2017). Cis-regulatory elements (CRE) are in part responsible for regulating gene expression and have emerged as ideal regions to target via gene editing to generate a range of phenotypes (Swinnen et al., 2016). CRISPR/Cas9-induced changes in CRE generally results in spatial and temporal changes in gene expression resulting in changes in quantitative trait variation (Rodríguez-Leal et al. 2017; Swinnen et al. 2016). Using this approach, a range of fruit size in tomato was achieved through semi-random CRISPR-induced mutations in regulatory region of the CLAVATA (SICLV3) gene (Rodríguez-Leal et al., 2017). This approach can be applied to many species to generate allelic series for a variety of traits relevant to plant breeding. A similar approach has been applied to the protein coding regions to achieve directed evolution for engineering improved or new functions in plants (Butt et al., 2019).

Abiotic stress tolerance is governed by several genes and largely influenced by environmental factors which make it challenging to study (Bhat et al. 2016; Deshmukh et al. 2014) and very difficult to develop new cultivars using conventional methods. CRISPR/Cas9 has potential to alter the stress response of crop plants. Shi et al. (2017) have developed a corn variety through CRISPR/Cas genome editing approach, which has improved yield under drought stress. In another study, a tissue-specific AtEF1 promoter was used to drive CRISPR/Cas9 induced mutations in abiotic stress-responsive genes, leading to enhanced stomatal responses (Osakabe and Osakabe 2017). Rice genes OsRR22 and OsNAC041 have also been targeted to increase salinity tolerance (Bo et al. 2019; Zhang et al. 2019). Regulatory sequences in promoters, such as W-box, GCC box (AGCCGCC), MYBR or DBS (TGCTANNATTG), which function as negative regulators of abiotic stress response by providing binding sites for transcription factors, could also be targeted by gene editing (Zafar et al., 2019).

Another approach to enhance abiotic stress tolerance is to modify the micro-structure of DNA through induced changes in DNA methylation. Reducing methylation of a gene greatly impacts its activity and function (Maurano et al. 2015). Therefore, it is possible to elicit gene expression by demethylating components of the promoter region. Many epigenetic factors, such as *H3K4me3* and *H3K9ac*, have been associated with abiotic stress tolerance, including heat and drought stress tolerance in maize, and for soil salinity tolerance in wheat (Varotto et al. 2020). These epigenetic factors represent potential targets for gene editing technologies for creating crop varieties adapted to specific environments. Alternatively, synthetic regulatory elements could be inserted into promoters to enhance expression of abiotic stress resistance genes, but this approach would require precise knowledge of specific genes that regulate the stress response.

4. Modulating Gene Recombination in Plants Using Gene Editing

Genetic recombination plays a foundational role in plant breeding as it allows for allele reshuffling and creates novel allelic combinations. It plays a critical role in introgression of a beneficial locus from a donor line into an elite line through backcrossing (Moose and Mumm, 2008). Recombination frequencies can be increased by using of chemical agents or physical stress, such as temperature shock or UV exposure (Wijnker and de Jong 2008), but results are often unpredictable. Ideally, backcrossing would result in a progeny containing just a small introgression from the donor line, but this is rarely the case, resulting in larger introgressions that may have a negative impact on one or several unrelated traits (often referred to as linkage drag). This is because recombination is not evenly distributed along chromosome and generally occurs in regions termed hotspots and is suppressed in other regions. For example, in wheat crossover events mainly occur at the distal region of both arms of the chromosomes while recombination is largely absent in the centromere proximal region (Choulet et al., 2014; Gardiner et al., 2019). Given that genetic recombination defines the amount of genetic diversity accessible to breeders, manipulation of recombination frequency is the focus of immense study. Because of its ability to target specific genomic regions and ability to generate double stranded DNA breaks (a prerequisite for genetic recombination), CRISPR/Cas gene editing is beginning to be used to

promote recombination at specific genomic regions (Filler Hayut et al., 2017; Sarno et al., 2017). In yeast, the left arm of chromosome 7 was targeted with 95 gRNAs to induce mitotic recombination (Sadhu et al., 2016). In tomato genomic sections of linked loci represent approximately 25% of the assembled genome (Lin et al., 2014). This is a prime example of where CRISPR/Cas based gene editing could be used for generation of recombinant individuals, generating diversity and breaking up these genetic linkages that would otherwise be not possible. However, work remains to identify the specific targets for editing to achieve the desired response.

5. Durable Crop Disease Resistance

Plants are constantly infested by a variety of pathogens, including viruses, bacteria, and fungi (Taylor et al. 2004), that can cause significant losses of crop quality and yield (Savary et al. 2012). Genome engineering technologies have been widely harnessed to engineer plant resistance against pathogens (Ali et al. 2015; Baltés et al. 2015; Iqbal et al. 2016; Ji et al. 2015). CRISPR/Cas9 and TALEN (transcription activator-like effector nucleases) were successfully used to generate resistance to powdery mildew by simultaneously targeting the three homologs of the *MILDEW-RESISTANCE LOCUS (MLO)*, *TaMLO-A*, *TaMLO-B*, and *TaMLO-D*, in wheat (Wang et al. 2014). The Tomelo transgene-free tomato, which is resistant to powdery mildew disease was developed by targeting the *SIMlo1* gene using CRISPR/Cas9 (Nekrasov et al. 2017). Simultaneous modification of the three homologs of the wheat *TaEDR1* gene enhanced resistance to powdery mildew disease (Zhang et al. 2017a). In other efforts, knockout of the ethylene-responsive factor (ERF) gene *OsERF922*, a negative regulator of rice blast resistance, enhanced resistance to the blast fungal pathogen (Wang et al. 2016a). Considerable knowledge has been accumulated on the genetic basis of plant disease resistance, and genes related to disease resistance have been identified in different plant species, including Arabidopsis, rice, soybean, potato and tomato (Hammond-Kosack and Jones 1996; Michelmore 1995). The wheat gene resistance allele *Lr34(res)* provides durable resistance against several pathogens, including two rust diseases, leaf rust and stripe rust, as well as powdery mildew and has been widely used in wheat breeding programs (Kolmer et al. 2008). The resistant allele of *Lr34(res)* differs from the susceptible allele

by genetic polymorphisms which change two amino acids in predicted transmembrane helices of an ABC transporter, a large family of genes in wheat (Krattinger et al. 2009; Risk et al. 2012). Fixing this allele using gene editing would greatly benefit subsequent breeding programs. Similarly, other members of the Lr family could be potential targets for gene editing technologies to produce plant immunity to various pathogens.

Pathogens exploit plants' susceptibility (S) genes to facilitate their proliferation (Pavan et al. 2009). Disrupting these S genes may interfere with the compatibility between the host and the pathogens and consequently provide broad-spectrum and durable disease resistance (Zaidi et al. 2018). Recent studies have demonstrated the effectiveness of new transgene-free gene editing technologies for deleting S genes in various economically important crops, including wheat (Shan et al. 2013; Wang et al. 2014), rice (Jiang et al. 2013; Li et al. 2012), tomato (Paula de Toledo Thomazella et al. 2016). Disruption of the S genes (*Mlo*, mildew resistance locus O) has conferred powdery mildew resistance in field for more than seven decades (van Schie and Takken 2014). Mutation of *Mlo* with CRISPR/Cas9 has also conferred powdery mildew resistance in wheat (Nekrasov et al. 2017; Shan et al. 2013; Wang et al. 2014) and tomato (Nekrasov et al. 2017). Another powdery mildew susceptibility locus, enhanced disease resistance 1 (*EDR1*), has been targeted by CRISPR/Cas9 and resulted in significant reduction of powdery mildew in wheat (Zhang et al. 2017b). Taken together these studies illustrate the efficacy of gene editing platforms in the development of disease-resistant crop varieties by introducing site-specific mutations to disrupt S genes in a transgene-free manner.

Plant pathogens can evolve rapidly in agriculture, especially in the presence of genetically uniform species and monocultures grown on a large scale, resulting in the break down of resistance that has already been deployed in commercial varieties. Breeders of all crop kinds must stay "ahead of the pathogen" by identifying multiple sources of effective resistance that are then combined or "stacked" together in an elite variety. In some instances however, only a single known effective resistance gene is available. For example, antibiosis resistance to the orange wheat blossom midge of wheat is conferred by the *Sm1* gene. *Sm1* is the only described OWBM resistance gene (Thomas et al. 2005), and efforts to identify and incorporate additional sources of resistance are ongoing (Thambugula et al. 2021). If *Sm1* resistance were to

breakdown, gene editing could be used to introduce “soft”, single-point mutations that could partially, or fully restore the resistance function, a process referred to R-gene recovery (Jang et al. 2020). Indeed, this would require substantial understanding of the *Sm1*, its function in the wheat plant in sensing that insect and initiating a defensive response. Fortunately, the *Sm1* gene was recently cloned (Walkowiak et al. 2020), providing an opportunity to understand the functional basis of resistance in wheat.

6. Reducing Gluten Immunogenicity AND IMPROVING GRAIN QUALITY in Wheat

Whole grain foods, including wheat, that contain all parts of the grain (i.e., the bran, starchy endosperm, and the germ) are known for their health benefits, reducing the risk of several non-communicable diseases (Ross et al. 2017; Zong et al. 2016). However, the ingestion of gluten proteins (gliadins and glutenins) from wheat, barley and rye can cause coeliac disease (CD) in genetically predisposed individuals (Gujral et al. 2012). CD leads to malnutrition and various related symptoms, ranging from bowel disorders to skin-, bone-, nerve-, and muscle-problems. The only way to prevent CD is a gluten-free (GF) diet, requiring complete exclusion of wheat, barley and rye. This is very difficult to adhere to, as gluten (especially from wheat) is added to many processed food products due to its viscoelastic and binding properties (Atchison et al. 2010).

Bread wheat contains two groups of gluten proteins: glutenins and gliadins. Both gliadins and glutenins contain immunogenic epitopes within their protein sequences that cause CD (Mitea et al. 2010a; Salentijn et al. 2009). So far, no food processing or breeding strategies have been developed that produce wheat-based food products that approach safety for CD patients (Boukid et al. 2017; Jouanin et al. 2018; Rustgi et al. 2019), although there is a clear need to do so (García-Molina et al. 2017; Ribeiro et al. 2018). With the ultimate goal of removing the immunogenic gluten epitopes from the human diet, CRISPR/Cas9 technology is being used in the development of wheat lines with fewer gluten genes and/or gluten genes with inactivated CD epitopes. As proof of concept, CRISPR/Cas9 technology has been used to edit α -gliadin genes (Sánchez-León et al. 2018) as well as both α - and γ -gliadin genes (Jouanin et al. 2019a; Jouanin et al. 2019b; Jouanin et al. 2020) in bread wheat. Along with ω -gliadins, these gliadin types rank

highest in abundance and overall immunogenicity compared with the low molecular weight (LMW) and high molecular weight (HMW) glutenins (Sollid et al. 2012; Tye-Din et al. 2010). α - and ω -epitopes are highly homologous (Dahal-Koirala et al. 2019; Tye-Din et al. 2010). With CRISPR/Cas9 it is possible to edit all gliadin epitopes by causing local deletions and frameshifts. However, after the first round of edits, modifying the few remaining epitopes in some of the genes would require multiple rounds of additional edits. Specific amino acid substitutions in a gliadin epitope can abolish its immunogenicity (Mitea et al. 2010b) while having no effect on gene expression and thus on bread dough quality.

Depending on intended end-use, gene editing could be used to develop a grain quality package consisting of multiple desirable alleles. For example, grain hardness can be addressed by editing the puroindoline-A and puroindoline-B (PIN) genes (Nadolska-Orczyk et al., 2009; Matus-Cadez et al. 2008). Wheat protein content can be improved by a single base pair edit in the TaNAM-B1 gene, a NAC transcription factor found in the Gpc-B1 locus (Uauy et al., 2006). Fixing these alleles through gene editing would reduce or eliminate the need for selection for these desirable alleles.

7. Gene Editing for Improved Quality of Barley

In Canada, about eight million tonnes of barley are produced each year. It is used for human food, livestock feed, and as malt to brew beer. One of the more beneficial substances in barley is beta-glucan (Limberger-Bayer et al. 2014). Barley cultivars with high grain beta-glucan content are preferred by the food sector because of its beneficial effect on human health (Cavallero et al. 2002; Collins et al. 2010). The beta-glucan acts as fermentable dietary fibre that reduces the risk of diet-related conditions such as cardiovascular disease, type II diabetes and colorectal cancer (Ames et al. 2019; Cosola et al. 2017) and contributes to lower cholesterol (Sima et al. 2018). However, the brewing and distilling industries require barley cultivars with low grain beta-glucan content for efficient malting and brewing (Bamforth and Martin 1983; Izydorczyk and Dexter 2008). This is because beta-glucan content has a direct impact on the viscosity of the mash and if too high, leads to filtration problems during brewing or to the formation of undesirable hazes in the final product (Gupta et al. 2010).

Scientists have used CRISPR/Cas9 technology to change beta-glucan levels in barley (Garcia-Gimenez et al. 2020). The authors used reverse genetics approach to generate changes in members of the gene superfamily responsible for making beta-glucan. Not only this approach allows improving grain quality, but researchers can also introduce some specific changes that would be desirable for specific industries that use barley. In other words, plant breeders will be able to create specialized barley cultivars for beer, bread, and other uses. The lignocellulosic residues of barley have the potential to be used as a feedstock for various purposes, including biofuel production. However, its heterogeneous properties and intrinsic recalcitrance caused by cell wall lignification have lowered the biorefinery efficiency. Lee et al. (2021) were able to reduce lignin content in barley by CRISPR/Cas9-mediated mutagenesis of *caffeic acid O-methyltransferase 1 (HvCOMT1)*, the lignin biosynthetic gene responsible for lignin formation. The mutant had 14% lower total lignin content and 34% higher fermentable glucose recovery rate, compared to the wild type. Thus, the transgene-free *HvCOMT1* mutant barley could offer improved quality lignocellulosic feedstock for efficient lignocellulosic biofuel production.

8. INCREASED Nutritional Value of Canola Oil and Seeds, using CRISPR/Cas9.

Since their domestication, Brassica oilseed species have undergone progressive transformation in parallel with breeding and molecular technologies. The canola (*Brassica napus*) crop has rapidly expanded globally in the last 30 years with intensive innovations in canola varieties, providing for a wider range of markets. Canola is the second most important oilseed crop in the world, ranking only behind soybean in production and value (Foreign Agricultural Service/USDA, 2020). Breeding efforts of canola have been mainly focused on improving seed yield, oil quality, and meal quality along with disease resistance, abiotic stress tolerance, and herbicide resistance (Ton et al. 2020). The parental lines for rapeseed breeding programs varied depending on the geographic location, with the spring type being widely cultivated in Canada, Australia, and northern Europe, the winter type being predominant in Asia and the remaining area of Europe (Heslop-Harrison 2013), and semi-winter type as the primary rapeseed in China (Wei et al. 2017).

In Canola, the proportions of the unsaturated fatty acids, oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) are impacted by fatty acid desaturase 2 (FAD2) and FAD3.

Recently, scientists used genomic editing technology based on CRISPR/Cas9 system to mutate all the copies of fatty acid genes (*BnaFAD2*), creating novel allelic variations in oleic acid and other fatty acid levels (Huang et al. 2020). Thus, new canola varieties with high oleic acid content, which has tremendous health benefits including a reduction of cardiovascular disease (Jones et al. 2014). On the other hand, canola protein isolate has been suggested as an alternative to other proteins for human food due to a balanced amino acid profile (Tan et al. 2011). Nonetheless, canola seeds contain a high proportion of phytic acid, which is considered as anti-nutritive for monogastric animals including humans due to its adverse effects on essential mineral absorption (Anwar et al. 2015; Wickramasuriya et al. 2015). CRISPR/Cas9 technology was successfully used to knock out genes controlling the accumulation of phytic acid in canola seeds (Sashidhar et al. 2020), thereby improving the nutritional value of canola seeds.

9. CRISPR/Cas9 for Improved Soybean Oil Quality and Early Flowering

Soybean (*Glycine max*) is an important crop with abundant sources of protein and oil for human food and animal feed. Traditional soybean breeding strategies are insufficient to cope with the increasing demands for soybean products in challenging environment fluctuations. Therefore, it is essential to introduce fast, accurate and efficient breeding strategies to cultivate better varieties, especially those with traits involved in yield, quality and stress tolerance or resistance (Gao 2018).

Since the completion of soybean genome sequencing project, a major challenge and research priority facing soybean researchers is to characterize the function of 46-56 thousand annotated genes (Bai et al. 2020). Transgenic technology is a valuable method for functional genomic research and genetic improvement of crop, but its application in soybean has been hindered by the recalcitrant nature of soybean to transformation via *Agrobacterium*. Fortunately, new genome editing technology has brought opportunities to address above challenges. Using CRISPR/Cas9 technology, scientists recently mutated two genes (*GmFAD2-1A* and *GmFAD2-1B*) to produce a high oleic, low linoleic and α -linolenic acid phenotype in soybean (Do et al. 2019). Similarly, in previous study, scientists (Demorest et al. 2016) reduced the polyunsaturated fats

by editing the genes *FAD2-1A* and *FAD2-1B*, using another gene editing technique, TALEN (transcription activator-like effector nucleases).

Soybean is a long-season crop, and as a result it has narrow geographical adaptive region. Using CRISPR/Cas9 technology, scientists generated precisely targeted mutations in *E1* gene controlling soybean flowering (Han et al. 2019). The truncated E1 protein in resulting mutants disinhibited *GmFT2a/5a* leading to their increased expressions, resulting in early flowering. Practical applications of new gene editing technologies to soybean breeding are slowly emerging. A gene edited soybean oil with an altered fatty acid profile has just hit the market in USA (<https://www.the-scientist.com/news-opinion/gene-edited-soybean-oil-makes-restaurant-debut-65590>).

10. Improving Fiber Quality and Seed Oil Composition in Flax using Gene Editing

Flax (*Linum usitatissimum*) is grown for its cellulose-rich fibers and seeds, which can be ground into a meal or turned into linseed oil. The long bast fibers are traditionally used in the textile, automobile, and construction industries (Baley et al. 2006; Kymäläinen and Sjöberg 2008). The use of plant fibers in various innovative fields has become very popular as they are environmentally friendly and biodegradable. Each fiber-consuming industry has special demands on the properties of fiber, which depend on morphological and anatomical characteristics, as well as on the structure and proportions of fiber bundle chemical constituents (Akin 2013). To manipulate these quality parameters, it is necessary to understand their genetic basis, and identify the genes controlling the target traits. Recently, RNA sequencing analysis identified 2666 genes with bast fiber-specific expression in flax (Mokshina et al. 2020). Proteins of the TIR-NBS-LRR class that are encoded by genes with fiber-specific expression, *LusRAD-6* transcription factor, as well as the genes involved in cellulose biosynthesis (*LusFLAs*, *LusLTPs*, *LusEXPA8*, and *LusKIN14H*), and RG-I metabolism (*LusRGL6* and *LusGT106*) were suggested as the most obvious candidates for general fiber development (irrespective of the stage) and for fiber cell wall thickening. These genes may serve as a target for molecular-genetic modulation of their expression. Using CRISPR/Cas9 technique, scientists could exclusively modulate bast fiber development, without influencing other tissues.

On the other hand, the wide use of flax seed oil is hindered by its high content of polyunsaturated fatty acids, particularly linolenic acid (18:3), which accounts for 30%–40% of seed oil (Shim et al. 2014). This compound makes flax oil more susceptible to oxidation and food products derived from this oil are more prone to rancidity (Fröhlich and Rice 2005). To address this deficiency in flax oil quality, efforts have been focused on increasing the content of the more oxidatively stable oleic acid by suppression of FAD2 genes for the D12 oleic acid desaturase that converts oleic acid to linoleic acid (18:2) and linolenic acid (18:3) (Hutcheon et al. 2010; Kang et al. 2011; Nguyen et al. 2013). This genetic modification increased oleic acid content while decreasing polyunsaturated fatty acid (18:2 and 18:3) content of seed oils (Nguyen et al. 2013). The recent advent of the highly efficient and facile CRISPR/Cas9 system for gene editing offers the opportunity to determine whether the oil composition of flax seeds could be favourably altered by knocking out the activities of a few or all of the six fatty acid desaturase 2 (FAD2) genes present in the genome of this allohexaploid plant (Hutcheon et al. 2010; Kang et al. 2011). If successful, this strategy would increase oleic acid content and lower the content of linoleic acid, linolenic acid and other long-chain polyunsaturated fatty acids.

11. Gene Editing could improve Maize Salt tolerance and Lodging Resistance.

Maize is an important crop worldwide, providing more than one-half of global calorie consumption (Schnable 2015). However, the global maize production is increasingly being challenged by diverse environmental stresses (Deinlein et al. 2014; Zuo et al. 2015), including soil salinity stress (Farooq et al. 2015). Soil salinity causes tremendous crop yield losses worldwide because high level of Na⁺ (salt) results in nutrient deficiency, retarded growth, and cell death. Soil salinization is commonly associated to land clearing by removal of deep root vegetation, thus accumulating more water and consequently raising the level of salty groundwater (Almeida et al. 2017).

Maize salt tolerance is a complex trait of distinct mechanisms (Munns and Tester 2008; Zhu 2016), occurring at cellular, subcellular and organ levels. In particular, under salt-affected soils the plant releases the excess of salt through its leaves to balance the potassium/sodium (K⁺/Na⁺) ratio, which maintains the plant healthy. This adaptative mechanism is controlled by

the up and downregulation of genes. Genetic and molecular analysis identified the gene *ZmNC1* (*ZmHKT1*) as a major player in the regulation of maize leaf Na⁺ extrusion and salt tolerance (Zhang et al. 2018a). Therefore, the application of new gene editing techniques (e.g., CRISPR/Cas9) to *ZmNC1* would help creating maize genotypes with improved salt tolerance. Similarly, the gene *Zm00001d039279* was identified recently (Sandhu et al. 2020) and was proposed to be a strong target for gene editing for enhanced salt tolerance. Rodríguez-Kessler (2006) found that two genes, *Zmodc* and *Zmspds2A*, are involved in salinity tolerance in maize roots through the accumulation of polyamine and spermidine. The expression of these genes could also be modulated through gene-editing techniques to prevent the harmful effects of soil salinization and avoid yield losses.

Semi dwarf plants can greatly contribute to crop improvement, as reported for semi dwarf “green revolution” rice (Sasaki et al. 2002) and wheat (Peng et al. 1999). The CRISPR/Cas9-mediated genome editing tool was utilized to edit the endogenous maize gene *ZmGA20ox3* (*GRMZM2G368411*), which resulted in semi dwarf phenotypes improving lodging resistance (Zhang et al. 2020). Hu et al. (2019) used the same technique to edit the *Semi-Dwarf1* (*SD1*) gene in two elite landraces, which contain many desired agronomic traits such as tolerance to low phosphorous and broad-spectrum resistance to several diseases and insects. Mutations of *SD1* confer shorter plant height for better resistance to lodging. Field trials demonstrated that the yield of the new lines was better than that of the wild type under modern cultivation and that the lines maintained the same desirable agronomic characteristics as their wild-type progenitors.

12. Tomato with Improved Bioactive Components

Lycopene is considered as a bioactive component for treating chronic diseases and lowering the risk of cancer and cardiovascular diseases (Li and Xu 2014; Pouchieu et al. 2014). Modulation of the expression of key genes in the lycopene metabolism pathway is an effective way to increase lycopene content. Most of studies on enhancing lycopene accumulation in tomato fruit by regulating the carotenoid metabolic pathway were mainly focused on the modification of individual genes, including a null mutation of the gene lycopene β-cyclase 2 (*LCY-B2*) (Ronen et

al. 2000), overexpression of the phytoene synthase 1 gene (*PSY1*) (Fraser et al. 2007), suppression of the 9-*cis*-epoxycarotenoid dioxygenase 1 (*NCED1*) gene (Sun et al. 2012) and the silencing of stay-green 1 (*SGR1*) (Luo et al. 2013). This process is laborious and time consuming.

Recently, multiplex CRISPR/Cas9 editing was successfully applied to regulate multiple genes associated with the carotenoid metabolic pathway of tomato, to increase the lycopene content (Li et al. 2018c). Sites were designed to target *SGR1* for promoting the synthesis of lycopene, whereas *LCY-E*, *LCY-B1*, and *LCY-B2* for catalyzing cyclisation of lycopene, and *Blc*. *LCY-E* to prevent the cyclisation from lycopene to α -carotene, and *LCY-B1* and *LCY-B2* to prevent the cyclisation from lycopene to β -carotene (Moreno et al. 2013; Ralley et al. 2016). This study provides the basis for acquiring new tomato varieties with improved agricultural traits. Japanese government has just approved a gene-edited 'super tomato' which contains four to five times more gamma-aminobutyric acid (GABA) than a regular tomato, an amino acid believed to aid relaxation and help lower blood pressure (<https://www.isaaa.org/kc/cropbiotechupdate/>).

After the fruit reaches the optimum edible stage, it will slowly deteriorate and lose its quality. Therefore, the regulation of fruit ripening has become the focus of many studies (Martín-Pizarro and Posé 2018). Many ripening genes have been edited with CRISPR/Cas9, including *RIN* (Ito et al. 2017; Ito et al. 2015), *IncRNA1459* (Li et al. 2018a), *SIORRM4* (Yang et al. 2017), and *SIDML2* (Zhou et al. 2019). All these results pave the way for creating new varieties in which fruits will remain firm for relatively long time while preserving their organoleptic properties.

13. Preserving Organoleptic Properties in Potato and Eggplant

Polyphenol Oxidases (PPOs) catalyze the conversion of phenolic substrates to quinones, leading to the formation of dark-colored precipitates in fruits and vegetables (Mayer 2006). This process, known as enzymatic browning, is the cause of undesirable changes in organoleptic properties and the loss of nutritional quality in plant-derived products (Jukanti 2017). In potato (*Solanum tuberosum* L.), enzymatic browning is a serious problem for both, producers and the industry, because the tubers can be affected during harvest and post-harvest procedures such as shipping, storage, distribution and blanching (Bachem et al. 1994).

In potato, PPOs are encoded by a multi-gene family with different expression patterns. A genome-wide survey revealed nine *StPPO*-like genes (named *StPPO1* to *9*), with differential prevalence of ESTs found from different potato tissues (Chi et al. 2014). Recently, the CRISPR/Cas9 system was applied to induce mutations in the *StPPO2* gene, responsible for most of the PPO activity and enzyme content in tubers (Chi et al. 2014). Interestingly, this gene editing technique also led to the elimination of larger, specific fragments from the coding sequence as was previously reported in potato (Tuncel et al. 2019; Veillet et al. 2019).

Eggplant (*Solanum melongena* L.) berries are characterized by a remarkable content of phenolic compounds, represented mainly by chlorogenic acid (5-O-caffeoylquinic acid). Chlorogenic acid plays important therapeutic roles due to its antioxidant, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory and anti-microbial properties (Naveed et al. 2018). However, in commercial varieties, the selection for berries with a reduced degree of browning in the flesh has resulted in the indirect selection of accessions with lower concentrations of phenolics (Jaime et al. 2007). Recently, scientists used CRISPR/Cas9 gene editing to simultaneously induce mutations in three *PPO* genes, *SmeIPPO4*, *SmeIPPO5* and *SmeIPPO6*, which resulted in reduced fruit flesh browning while preserving the content of phenolics (Maioli et al. 2020). This work opens the way to the development of eggplant genotypes with low flesh browning while maintaining a high polyphenol content in the berries.

14. Vicine-free Faba bean Cultivars

Faba bean (*Vicia faba* L.) is an excellent source of plant-based protein (Crépon et al. 2010) and provides a balanced diet of lysine-rich protein, carbohydrates, fibre and phytochemicals (Köpke and Nemecek 2010). However, its use as a food crop has been restricted, primarily due to the presence of glycosides vicine and convicine (v-c) in the seed. Ingestion of v-c can cause favism in some individuals with a genetically inherited deficiency (Luzzatto and Arese 2018). Levels of v-c can be reduced by soaking the seeds in a weak acid solution prior to cooking, by boiling, roasting or microwave irradiation, or via alkaline extraction with acid precipitation. Genetic approaches to reducing v-c levels are challenging given that faba bean is an outcrossing species. Indeed germplasm has been identified that has a 95% reduction in v-c levels, but the biosynthetic

pathway responsible for v-c levels is still lacking (Khazaei et al. 2019). Breeding efforts to reduce v-c contents are ongoing, but a near zero level of v-c has not yet been achieved with conventional breeding and processing methods (Crépon et al. 2010; Pulkkinen et al. 2015).

The genetic analysis of v-c production in faba bean supports a single major gene (Khazaei et al. 2015). Recent studies have elucidated the biosynthetic pathway for the pyrimidine glycosides vicine convicine (Björnsdotter et al. 2020). The bifunctional riboflavin biosynthesis protein RIBA1, which is now termed *VC1*, catalyses a key step in v-c biosynthesis. Editing this gene with a targeted single DNA base change should be sufficient to generate faba bean cultivars that are nearly free from these anti-nutrients, providing a safe and sustainable source of dietary protein. Since there are several faba bean cultivars already available that express suitable agronomics, gene editing would provide a rapid approach to convert these cultivars to low v-c types.

15. Eliminating the off-flavour of pea protein

Canada leads the world in dry pea production with a global share of 32%. As the market for alternative sources of proteins is expanding rapidly, pea has received much attention as a potential leading source of plant-based proteins. Major industry players, such as Roquette, Cargill and Burcon are investing nearly half a billion dollars in the construction of pea processing facilities. Thus, there is great potential for further economic growth in Canadian pea. Pea protein isolates and concentrates are key ingredients in diverse plant-based foods. Despite the positive consumer sentiment, the adoption of pea as a mainstream source of proteins is affected by the characteristic “off-flavors” often described as green, beany, hay-like, metallic, and astringent. The off-flavors are caused by a mixture of volatile organic compounds, such as methoxypyrazines, alcohols, ketones, aldehydes, etc. and non-volatile saponins. The production of volatile compounds in pea is initiated by the conversion of polyunsaturated fatty acids into hydroperoxide forms of fatty acids, mainly catalyzed by lipoxygenases (LOX). A null mutant of *LOX2* has been shown to produce decreased levels of volatile organic compounds with no compromises in seed yield and weight (Forster et al., 1999). The LOX genes are obvious targets for CRISPR/Cas9 gene editing. The non-volatile saponins are a group of amphipathic compounds

comprised of lipophilic triterpene and hydrophilic sugar moieties. The biosynthetic genes for saponins have been reported in the literature (e.g., β -amyrin synthase, C22 hydroxylase, UDP-glycosyltransferase; Morita et al., 2000; Sundaramoorthy et al., 2019). CRISPR/Cas9 gene editing can be used to remove or alter the levels of saponins by creating null mutants of its biosynthesis genes.

Summary

Domestication and plant breeding has led to high yielding crop varieties which are adapted to local growing conditions. However, the growing human population faces a number of agricultural challenges including climate change, changes in abiotic stress and biotic stress along with loss of arable land and a demand for more sustainable and precise agricultural practices. Many crop traits have been fixed through initial waves of domestication, and in this review, we discussed several possibilities to generate another wave of important traits. CRISPR/Cas based gene editing provides a means by which we can create naturally occurring allelic variants without the constraints of traditional introgression breeding. In addition, we can now create new desirable genetic variants and counteract some of the loss of allelic diversity due to selective breeding. However, gene-editing should not be interpreted as a replacement for plant breeding. It is a tool of plant breeding, in that the technology generates genetic variation to which selection is applied. Indeed, the variation induced by gene-editing may not be the only strategy available to the plant breeder, nor will it negate the need for the plant breeder to evaluate the phenotypic outcome, or the stability of trait expression in a range of environments.

There still remains some challenges for routine application of gene editing in plant breeding. One of the biggest hurdles for the application of plant gene editing technologies is the need for a repeatable delivery method into totipotent cells, which could be widely applied to diverse plant species, especially recalcitrant species like pulse crops. Secondly, studies have shown that gene editing can lead to “off-target” mutations. However, as gene-editing technology has evolved, improvements are being seen in specificity, precision and off-target effects, editing capabilities, and ease of use in target organisms. Thirdly, functional genome annotation is critical to the success of gene editing experiments. Functional genomics research annotates the “role” of each

gene and it is necessary to bridge the gap between genotype and phenotype. Functional annotation is in most cases, a prerequisite to gene editing experiments. It allows selection of gene targets for editing that will have a high probability to impact trait response. It also provides clues as to the impact gene editing may have on gene function. Fortunately, the genome sequences of many important agricultural crops have become available, and functional annotation is becoming more routine. Gene editing provides an exciting opportunity to blend functional gene characterization with applied plant breeding. Lastly, the regulatory framework surrounding gene edited plant lines will impact how and where this technology is realized. Regulations that promote the use of gene editing are emerging in several countries, which will pave the way for programs to design strategies for optimal use of the technology to support cultivar development.

References

Akin DE (2013) Linen Most Useful: Perspectives on Structure, Chemistry, and Enzymes for Retting Flax. *ISRN Biotechnology* 2013:186534

Ali Z, Abul-faraj A, Li L, Ghosh N, Piatek M, Mahjoub A, Aouida M, Piatek A, Baltes NJ, Voytas DF, Dinesh-Kumar S, Mahfouz MM (2015) Efficient Virus-Mediated Genome Editing in Plants Using the CRISPR/Cas9 System. *Mol Plant* 8:1288-1291

Almeida DM, Oliveira MM, Saibo NJM (2017) Regulation of Na⁺ and K⁺ homeostasis in plants: towards improved salt stress tolerance in crop plants. *Genet Mol Biol* 40:326-345

Altpeter F, Springer NM, Bartley LE, Blechl AE, Brutnell TP, Citovsky V, Conrad LJ, Gelvin SB, Jackson DP, Kausch AP, Lemaux PG, Medford JI, Orozco-Cárdenas ML, Tricoli DM, Van Eck J, Voytas DF, Walbot V, Wang K, Zhang ZJ, Stewart CN (2016) Advancing Crop Transformation in the Era of Genome Editing. *The Plant cell* 28:1510-1520

Ames N, Storsley J, Thandapilly SJ (2019) CHAPTER 8 Functionality of Beta-glucan from Oat and Barley and Its Relation with Human Health. *Cereal Grain-based Functional Foods: Carbohydrate and Phytochemical Components*. The Royal Society of Chemistry, pp 147-166

Anwar MM, Ali SE, Nasr EH (2015) Improving the nutritional value of canola seed by gamma irradiation. *Journal of Radiation Research and Applied Sciences* 8:328-333

Ashokkumar S, Jaganathan D, Ramanathan V, Rahman H, Palaniswamy R, Kambale R, Muthurajan R (2020) Creation of novel alleles of fragrance gene *OsBADH2* in rice through CRISPR/Cas9 mediated gene editing. *PloS one* 15:e0237018

Atchison J, Head L, Gates A (2010) Wheat as food, wheat as industrial substance; comparative geographies of transformation and mobility. *Geoforum* 41:236-246

Bachem CWB, Speckmann G-J, van der Linde PCG, Verheggen FTM, Hunt MD, Steffens JC, Zabeau M (1994) Antisense Expression of Polyphenol Oxidase Genes Inhibits Enzymatic Browning in Potato Tubers. *Bio/Technology* 12:1101-1105

Bai M, Yuan J, Kuang H, Gong P, Li S, Zhang Z, Liu B, Sun J, Yang M, Yang L, Wang D, Song S, Guan Y (2020) Generation of a multiplex mutagenesis population via pooled CRISPR-Cas9 in soya bean. *Plant Biotechnology Journal* 18:721-731

Baley C, Perrot Y, Busnel F, Guezenoc H, Davies P (2006) Transverse tensile behaviour of unidirectional plies reinforced with flax fibres. *Materials Letters* 60:2984-2987

Baltes NJ, Hummel AW, Konecna E, Cegan R, Bruns AN, Bisaro DM, Voytas DF (2015) Conferring resistance to geminiviruses with the CRISPR-Cas prokaryotic immune system. *Nature Plants* 1:15145

Bamforth CW, Martin HL (1983) THE DEGRADATION OF β -GLUCAN DURING MALTING AND MASHING: THE ROLE OF β -GLUCANASE. *Journal of the Institute of Brewing* 89:303-307

Basma AN, Morris EJ, Nicklas WJ, Geller HM (1995) L-DOPA Cytotoxicity to PC12 Cells in Culture Is via Its Autoxidation. *Journal of Neurochemistry* 64:825-832

Bhat JA, Ali S, Salgotra RK, Mir ZA, Dutta S, Jadon V, Tyagi A, Mushtaq M, Jain N, Singh PK, Singh GP, Prabhu KV (2016) Genomic Selection in the Era of Next Generation Sequencing for Complex Traits in Plant Breeding. *Frontiers in genetics* 7:221

Björnsdotter E, Nadzieja M, Chang W, Escobar-Herrera L, Mancinotti D, Angra D, Khazaei H, Crocoll C, Vandenberg A, Stoddard FL, O'Sullivan DM, Stougaard J, Schulman AH, Andersen SU, Geu-Flores F (2020) VC1 catalyzes a key step in the biosynthesis of vicine from GTP in faba bean. [bioRxiv:2020.2002.2026.966523](https://doi.org/10.1101/2020.2002.2026.966523)

Bo W, Zhaohui Z, Huanhuan Z, Xia W, Binglin L, Lijia Y, Xiangyan H, Deshui Y, Xuelian Z, Chunguo W, Wenqin S, Chengbin C, Yong Z (2019) Targeted Mutagenesis of NAC Transcription Factor Gene, OsNAC041, Leading to Salt Sensitivity in Rice. *Rice Science* 26:98-108

Boukid F, Mejri M, Pellegrini N, Sforza S, Prandi B (2017) How Looking for Celiac-Safe Wheat Can Influence Its Technological Properties. *Comprehensive reviews in food science and food safety* 16:797-807

Boyko A, Kovalchuk I (2008) Epigenetic control of plant stress response. *Environmental and molecular mutagenesis* 49:61-72

Campa CC, Weisbach NR, Santinha AJ, Incarnato D, Platt RJ (2019) Multiplexed genome engineering by Cas12a and CRISPR arrays encoded on single transcripts. *Nature Methods* 16:887-893

Cardoza V, Stewart CN (2004) Agrobacterium-Mediated Transformation of Canola. In: Curtis IS (ed) *Transgenic Crops of the World: Essential Protocols*. Springer Netherlands, Dordrecht, pp 379-387

Cavallero A, Empilli S, Brighenti F, Stanca AM (2002) High (1 \rightarrow 3,1 \rightarrow 4)- β -Glucan Barley Fractions in Bread Making and their Effects on Human Glycemic Response. *Journal of Cereal Science* 36:59-66

Charlesworth D, Willis JH (2009) The genetics of inbreeding depression. *Nature Reviews Genetics* 10:783-796

Chi M, Bhagwat B, Lane WD, Tang G, Su Y, Sun R, Oomah BD, Wiersma PA, Xiang Y (2014) Reduced polyphenol oxidase gene expression and enzymatic browning in potato (*Solanum tuberosum* L.) with artificial microRNAs. *BMC Plant Biology* 14:62

Collins HM, Burton RA, Topping DL, Liao M-L, Bacic A, Fincher GB (2010) REVIEW: Variability in Fine Structures of Noncellulosic Cell Wall Polysaccharides from Cereal Grains: Potential Importance in Human Health and Nutrition. *Cereal Chemistry* 87:272-282

Cosola C, De Angelis M, Rocchetti MT, Montemurno E, Maranzano V, Dalfino G, Manno C, Zito A, Gesualdo M, Ciccone MM, Gobbetti M, Gesualdo L (2017) Beta-Glucans Supplementation Associates with Reduction in P-Cresyl Sulfate Levels and Improved Endothelial Vascular Reactivity in Healthy Individuals. *PloS one* 12:e0169635

Crépon K, Marget P, Peyronnet C, Carrouée B, Arese P, Duc G (2010) Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Research* 115:329-339

Cubas P, Vincent C, Coen E (1999) An epigenetic mutation responsible for natural variation in floral symmetry. *Nature* 401:157-161

Dahal-Koirala S, Ciacchi L, Petersen J, Risnes LF, Neumann RS, Christophersen A, Lundin KEA, Reid HH, Qiao S-W, Rossjohn J, Sollid LM (2019) Discriminative T-cell receptor recognition of highly homologous HLA-DQ2-bound gluten epitopes. *J Biol Chem* 294:941-952

Deinlein U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI (2014) Plant salt-tolerance mechanisms. *Trends in plant science* 19:371-379

Demorest ZL, Coffman A, Baltus NJ, Stoddard TJ, Clasen BM, Luo S, Retterath A, Yabandith A, Gamo ME, Bissen J, Mathis L, Voytas DF, Zhang F (2016) Direct stacking of sequence-specific nuclease-induced mutations to produce high oleic and low linolenic soybean oil. *BMC Plant Biology* 16:225

Deshmukh R, Sonah H, Patil G, Chen W, Prince S, Mutava R, Vuong T, Valliyodan B, Nguyen HT (2014) Integrating omic approaches for abiotic stress tolerance in soybean. *Front Plant Sci* 5:244

Do PT, Nguyen CX, Bui HT, Tran LTN, Stacey G, Gillman JD, Zhang ZJ, Stacey MG (2019) Demonstration of highly efficient dual gRNA CRISPR/Cas9 editing of the homeologous GmFAD2-1A and GmFAD2-1B genes to yield a high oleic, low linoleic and α -linolenic acid phenotype in soybean. *BMC Plant Biology* 19:311

Farooq M, Hussain M, Wakeel A, Siddique KHM (2015) Salt stress in maize: effects, resistance mechanisms, and management. A review. *Agronomy for Sustainable Development* 35:461-481

Fernie AR, Yan J (2019) De Novo Domestication: An Alternative Route toward New Crops for the Future. *Molecular Plant* 12:615-631

Forster, C., North, H., Afzal, N., Domoney, C., Hornostaj, A., Robinson, D.S., and Casey, R. (1999). Molecular analysis of a null mutant for pea (*Pisum sativum* L.) seed lipoxygenase-2. *Plant molecular biology* 39, 1209-1220.

Fraser PD, Enfissi EMA, Halket JM, Truesdale MR, Yu D, Gerrish C, Bramley PM (2007) Manipulation of Phytoene Levels in Tomato Fruit: Effects on Isoprenoids, Plastids, and Intermediary Metabolism. *The Plant cell* 19:3194-3211

Fröhlich A, Rice B (2005) Evaluation of *Camelina sativa* oil as a feedstock for biodiesel production. *Industrial Crops and Products* 21:25-31

Gao C (2018) The future of CRISPR technologies in agriculture. *Nature Reviews Molecular Cell Biology* 19:275-276

Garcia-Gimenez G, Barakate A, Smith P, Stephens J, Khor SF, Doblin MS, Hao P, Bacic A, Fincher GB, Burton RA, Waugh R, Tucker MR, Houston K (2020) Targeted mutation of barley (1,3;1,4)- β -glucan synthases reveals complex relationships between the storage and cell wall polysaccharide content. [bioRxiv:2020.2005.2013.093146](https://doi.org/10.1101/2020.05.2013.093146)

García-Molina MD, Muccilli V, Saletti R, Foti S, Masci S, Barro F (2017) Comparative proteomic analysis of two transgenic low-gliadin wheat lines and non-transgenic wheat control. *Journal of proteomics* 165:102-112

Gaut BS, Seymour DK, Liu Q, Zhou Y (2018) Demography and its effects on genomic variation in crop domestication. *Nature Plants* 4:512-520

Gepts P (2014) The contribution of genetic and genomic approaches to plant domestication studies. *Current Opinion in Plant Biology* 18:51-59

Gujral N, Freeman HJ, Thomson ABR (2012) Celiac disease: prevalence, diagnosis, pathogenesis and treatment. *World J Gastroenterol* 18:6036-6059

Gupta M, Abu-Ghannam N, Gallagher E (2010) Barley for Brewing: Characteristic Changes during Malting, Brewing and Applications of its By-Products. *Comprehensive reviews in food science and food safety* 9:318-328

Gupta YP (1987) Anti-nutritional and toxic factors in food legumes: a review. *Plant Foods for Human Nutrition* 37:201-228

Hammond-Kosack KE, Jones JD (1996) Resistance gene-dependent plant defense responses. *The Plant cell* 8:1773-1791

Han J, Guo B, Guo Y, Zhang B, Wang X, Qiu L-J (2019) Creation of Early Flowering Germplasm of Soybean by CRISPR/Cas9 Technology. *Frontiers in Plant Science* 10

Heslop-Harrison P (2013) Genetics, genomics and breeding of oilseed brassicas. *Ann Bot* 112:vi-vi

Hillman GC, Davies MS (1990) 6. Domestication rates in wild-type wheats and barley under primitive cultivation. *Biological Journal of the Linnean Society* 39:39-78

Hu X, Cui Y, Dong G, Feng A, Wang D, Zhao C, Zhang Y, Hu J, Zeng D, Guo L, Qian Q (2019) Using CRISPR-Cas9 to generate semi-dwarf rice lines in elite landraces. *Scientific reports* 9:19096

Hua L, Wang DR, Tan L, Fu Y, Liu F, Xiao L, Zhu Z, Fu Q, Sun X, Gu P, Cai H, McCouch SR, Sun C (2015) LABA1, a Domestication Gene Associated with Long, Barbed Awns in Wild Rice. *The Plant cell* 27:1875-1888

Huang H, Cui T, Zhang L, Yang Q, Yang Y, Xie K, Fan C, Zhou Y (2020) Modifications of fatty acid profile through targeted mutation at BnaFAD2 gene with CRISPR/Cas9-mediated gene editing in *Brassica napus*. *Theoretical and Applied Genetics* 133:2401-2411

Hutcheon C, Ditt RF, Beilstein M, Comai L, Schroeder J, Goldstein E, Shewmaker CK, Nguyen T, De Rocher J, Kiser J (2010) Polyploid genome of *Camelina sativa* revealed by isolation of fatty acid synthesis genes. *BMC plant biology* 10:233-233

Iqbal Z, Sattar MN, Shafiq M (2016) CRISPR/Cas9: A Tool to Circumscribe Cotton Leaf Curl Disease. *Front Plant Sci* 7:475

Ito Y, Nishizawa-Yokoi A, Endo M, Mikami M, Shima Y, Nakamura N, Kotake-Nara E, Kawasaki S, Toki S (2017) Re-evaluation of the rin mutation and the role of RIN in the induction of tomato ripening. *Nature Plants* 3:866-874

Ito Y, Nishizawa-Yokoi A, Endo M, Mikami M, Toki S (2015) CRISPR/Cas9-mediated mutagenesis of the RIN locus that regulates tomato fruit ripening. *Biochemical and biophysical research communications* 467:76-82

Izydorczyk MS, Dexter JE (2008) Barley β -glucans and arabinoxylans: Molecular structure, physicochemical properties, and uses in food products—a Review. *Food Research International* 41:850-868

Jaime P, Adrián R-B, María Dolores R, Fernando N (2007) Total Phenolic Concentration and Browning Susceptibility in a Collection of Different Varietal Types and Hybrids of Eggplant: Implications for Breeding for Higher Nutritional Quality and Reduced Browning. *Journal of the American Society for Horticultural Science J Amer Soc Hort Sci* 132:638-646

Jang, HK., Song, B., Hwang, GH. *et al.* Current trends in gene recovery mediated by the CRISPR-Cas system. *Exp Mol Med* 52, 1016–1027 (2020). <https://doi.org/10.1038/s12276-020-0466-1>

Ji X, Zhang H, Zhang Y, Wang Y, Gao C (2015) Establishing a CRISPR–Cas-like immune system conferring DNA virus resistance in plants. *Nature Plants* 1:15144

Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP (2013) Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in *Arabidopsis*, tobacco, sorghum and rice. *Nucleic Acids Research* 41:e188-e188

Jones PJ, Senanayake VK, Pu S, Jenkins DJ, Connelly PW, Lamarche B, Couture P, Charest A, Baril-Gravel L, West SG, Liu X, Fleming JA, McCrea CE, Kris-Etherton PM (2014) DHA-enriched high-oleic acid canola oil improves lipid profile and lowers predicted cardiovascular disease risk in the canola oil multicenter randomized controlled trial. *The American Journal of Clinical Nutrition* 100:88-97

Jouanin A, Borm T, Boyd LA, Cockram J, Leigh F, Santos BACM, Visser RGF, Smulders MJM (2019a) Development of the GlutEnSeq capture system for sequencing gluten gene families in hexaploid bread wheat with deletions or mutations induced by γ -irradiation or CRISPR/Cas9. *Journal of Cereal Science* 88:157-166

Jouanin A, Gilissen L, Boyd LA, Cockram J, Leigh FJ, Wallington EJ, van den Broeck HC, van der Meer IM, Schaart JG, Visser RGF, Smulders MJM (2018) Food processing and breeding strategies for coeliac-safe and healthy wheat products. *Food research international (Ottawa, Ont)* 110:11-21

Jouanin A, Schaart JG, Boyd LA, Cockram J, Leigh FJ, Bates R, Wallington EJ, Visser RGF, Smulders MJM (2019b) Outlook for coeliac disease patients: towards bread wheat with hypoimmunogenic gluten by gene editing of α - and γ -gliadin gene families. *BMC Plant Biology* 19:333

Jouanin A, Tenorio-Berrio R, Schaart JG, Leigh F, Visser RGF, Smulders MJM (2020) Optimisation of droplet digital PCR for determining copy number variation of α -gliadin genes in mutant and gene-edited polyploid bread wheat. *Journal of Cereal Science* 92:102903

Jukanti A (2017) Distribution, Localization, and Structure of Plant Polyphenol Oxidases (PPOs). In: Jukanti A (ed) *Polyphenol Oxidases (PPOs) in Plants*. Springer Singapore, Singapore, pp 11-32

Kang J, Snapp AR, Lu C (2011) Identification of three genes encoding microsomal oleate desaturases (FAD2) from the oilseed crop *Camelina sativa*. *Plant physiology and biochemistry : PPB* 49:223-229

Khazaei H, O'Sullivan DM, Jones H, Pitts N, Sillanpää MJ, Pärssinen P, Manninen O, Stoddard FL (2015) Flanking SNP markers for vicine-convicine concentration in faba bean (*Vicia faba* L.). *Molecular Breeding* 35:38

Kolmer JA, Singh RP, Garvin DF, Viccars L, William HM, Huerta-Espino J, Ogbonnaya FC, Raman H, Orford S, Bariana HS, Lagudah ES (2008) Analysis of the Lr34/Yr18 Rust Resistance Region in Wheat Germplasm. *Crop Science* 48:1841-1852

Köpke U, Nemecek T (2010) Ecological services of faba bean. *Field Crops Research* 115:217-233

Krattinger SG, Lagudah ES, Spielmeier W, Singh RP, Huerta-Espino J, McFadden H, Bossolini E, Selter LL, Keller B (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. *Science (New York, NY)* 323:1360-1363

Kymäläinen H-R, Sjöberg A-M (2008) Flax and hemp fibres as raw materials for thermal insulations. *Building and Environment* 43:1261-1269

Lane EL (2019) L-DOPA for Parkinson's disease-a bittersweet pill. *The European journal of neuroscience* 49:384-398

Lee JH, Won HJ, Hoang Nguyen Tran P, Lee S-m, Kim H-Y, Jung JH (2021) Improving lignocellulosic biofuel production by CRISPR/Cas9-mediated lignin modification in barley. *GCB Bioenergy* 13:742-752

Lemmon ZH, Reem NT, Dalrymple J, Soyk S, Swartwood KE, Rodriguez-Leal D, Van Eck J, Lippman ZB (2018) Rapid improvement of domestication traits in an orphan crop by genome editing. *Nature Plants* 4:766-770

Li R, Fu D, Zhu B, Luo Y, Zhu H (2018a) CRISPR/Cas9-mediated mutagenesis of lncRNA1459 alters tomato fruit ripening. *The Plant Journal* 94:513-524

Li T, Liu B, Spalding MH, Weeks DP, Yang B (2012) High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nature Biotechnology* 30:390-392

Li T, Yang X, Yu Y, Si X, Zhai X, Zhang H, Dong W, Gao C, Xu C (2018b) Domestication of wild tomato is accelerated by genome editing. *Nature Biotechnology* 36:1160-1163

Li X, Wang Y, Chen S, Tian H, Fu D, Zhu B, Luo Y, Zhu H (2018c) Lycopene Is Enriched in Tomato Fruit by CRISPR/Cas9-Mediated Multiplex Genome Editing. *Frontiers in Plant Science* 9

Li X, Xu J (2014) Meta-analysis of the association between dietary lycopene intake and ovarian cancer risk in postmenopausal women. *Scientific reports* 4:4885

Limberger-Bayer VM, de Francisco A, Chan A, Oro T, Ogliari PJ, Barreto PLM (2014) Barley β -glucans extraction and partial characterization. *Food Chemistry* 154:84-89

Lowder LG, Zhang D, Baltus NJ, Paul JW, Tang X, Zheng X, Voytas DF, Hsieh T-F, Zhang Y, Qi Y (2015) A CRISPR/Cas9 Toolbox for Multiplexed Plant Genome Editing and Transcriptional Regulation. *Plant Physiology* 169:971-985

Luo Z, Zhang J, Li J, Yang C, Wang T, Ouyang B, Li H, Giovannoni J, Ye Z (2013) A STAY-GREEN protein SISGR1 regulates lycopene and β -carotene accumulation by interacting directly with SIPSY1 during ripening processes in tomato. *New Phytologist* 198:442-452

Luzzatto L, Arese P (2018) Favism and Glucose-6-Phosphate Dehydrogenase Deficiency. *New England Journal of Medicine* 378:60-71

Lyzenga W, Pozniak C, Kagale S (2021) Advanced domestication: harnessing the precision of gene editing in crop breeding. *Plant Biotechnol J.* doi: 10.1111/pbi.13576.

Maioli A, Gianoglio S, Moglia A, Acquadro A, Valentino D, Milani AM, Prohens J, Orzaez D, Granell A, Lanteri S, Comino C (2020) Simultaneous CRISPR/Cas9 Editing of Three PPO Genes Reduces Fruit Flesh Browning in *Solanum melongena* L. *Frontiers in plant science* 11:607161-607161

Manning K, Tör M, Poole M, Hong Y, Thompson AJ, King GJ, Giovannoni JJ, Seymour GB (2006) A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nat Genet* 38:948-952

Martín-Pizarro C, Posé D (2018) Genome Editing as a Tool for Fruit Ripening Manipulation. *Front Plant Sci* 9:1415

Matus-Cádiz MA, Pozniak CJ, Hucl P (2008) Puroindoline allele diversity in Canadian and northern US hard spring wheat varieties differing in kernel hardness. *Canadian Journal of Plant Science* 88:873-883

Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y, Turecki G, Delaney A, Varhol R, Thiessen N, Shchors K, Heine VM, Rowitch DH, Xing X, Fiore C, Schillebeeckx M, Jones SJM, Haussler D, Marra MA, Hirst M, Wang T, Costello JF (2010) Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* 466:253-257

Maurano MT, Wang H, John S, Shafer A, Canfield T, Lee K, Stamatoyannopoulos JA (2015) Role of DNA Methylation in Modulating Transcription Factor Occupancy. *Cell reports* 12:1184-1195

Mayer AM (2006) Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry* 67:2318-2331

Michelmore R (1995) Molecular Approaches to Manipulation of Disease Resistance Genes. *Annual Review of Phytopathology* 33:393-427

Mitea C, Salentijn EM, van Veelen P, Goryunova SV, van der Meer IM, van den Broeck HC, Mujico JR, Montserrat V, Gilissen LJ, Drijfhout JW, Dekking L, Koning F, Smulders MJ (2010a) A universal approach to eliminate antigenic properties of alpha-gliadin peptides in celiac disease. *PloS one* 5:e15637

Mitea C, Salentijn EMJ, van Veelen P, Goryunova SV, van der Meer IM, van den Broeck HC, Mujico JR, Monserrat V, Gilissen LJWJ, Drijfhout JW, Dekking L, Koning F, Smulders MJM (2010b) A Universal Approach to Eliminate Antigenic Properties of Alpha-Gliadin Peptides in Celiac Disease. *PloS one* 5:e15637

Miura K, Agetsuma M, Kitano H, Yoshimura A, Matsuoka M, Jacobsen SE, Ashikari M (2009) A metastable DWARF1 epigenetic mutant affecting plant stature in rice. *Proceedings of the National Academy of Sciences of the United States of America* 106:11218-11223

Mokshina N, Gorshkov O, Galinousky D, Gorshkova T (2020) Genes with bast fiber-specific expression in flax plants - Molecular keys for targeted fiber crop improvement. *Industrial Crops and Products* 152:112549

Moreno JC, Pizarro L, Fuentes P, Handford M, Cifuentes V, Stange C (2013) Levels of Lycopene β -Cyclase 1 Modulate Carotenoid Gene Expression and Accumulation in *Daucus carota*. *PLoS one* 8:e58144

Morita, M., Shibuya, M., Kushiro, T., Masuda, K., and Ebizuka, Y. (2000). Molecular cloning and functional expression of triterpene synthases from pea (*Pisum sativum*) New α -amyirin-producing enzyme is a multifunctional triterpene synthase. *European Journal of Biochemistry* 267, 3453-3460.

Mueller NG, Fritz GJ, Patton P, Carmody S, Horton ET (2017) Growing the lost crops of eastern North America's original agricultural system. *Nature Plants* 3:17092

Multari S, Stewart D, Russell WR (2015) Potential of Fava Bean as Future Protein Supply to Partially Replace Meat Intake in the Human Diet. *Comprehensive reviews in food science and food safety* 14:511-522

Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59:651-681

Nadolska-Orczyk A, Gasparis S, Orczyk W (2009) The determinants of grain texture in cereals. *Journal of applied genetics* 50:185-197

Naveed M, Hejazi V, Abbas M, Kamboh AA, Khan GJ, Shumzaid M, Ahmad F, Babazadeh D, FangFang X, Modarresi-Ghazani F, WenHua L, XiaoHui Z (2018) Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* 97:67-74

Nekrasov V, Wang C, Win J, Lanz C, Weigel D, Kamoun S (2017) Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. *Scientific reports* 7:482

Neves DM, Almeida LAdH, Santana-Vieira DDS, Freschi L, Ferreira CF, Soares Filho WdS, Costa MGC, Micheli F, Coelho Filho MA, Gesteira AdS (2017) Recurrent water deficit causes epigenetic and hormonal changes in citrus plants. *Scientific reports* 7:13684

Nguyen HT, Silva JE, Podicheti R, Macrander J, Yang W, Nazarens TJ, Nam JW, Jaworski JG, Lu C, Scheffler BE, Mockaitis K, Cahoon EB (2013) Camelina seed transcriptome: a tool for meal and oil improvement and translational research. *Plant Biotechnol J* 11:759-769

Nogué F, Mara K, Collonnier C, Casacuberta JM (2016) Genome engineering and plant breeding: impact on trait discovery and development. *Plant cell reports* 35:1475-1486

Osakabe Y, Osakabe K (2017) Chapter Six - Genome Editing to Improve Abiotic Stress Responses in Plants. In: Weeks DP, Yang B (eds) Progress in Molecular Biology and Translational Science. Academic Press, pp 99-109

Østerberg JT, Xiang W, Olsen LI, Edenbrandt AK, Vedel SE, Christiansen A, Landes X, Andersen MM, Pagh P, Sandøe P, Nielsen J, Christensen SB, Thorsen BJ, Kappel K, Gamborg C, Palmgren M (2017) Accelerating the Domestication of New Crops: Feasibility and Approaches. Trends in plant science 22:373-384

Park SJ, Jiang K, Tal L, Yichie Y, Gar O, Zamir D, Eshed Y, Lippman ZB (2014) Optimization of crop productivity in tomato using induced mutations in the florigen pathway. Nature Genetics 46:1337-1342

Paula de Toledo Thomazella D, Brail Q, Dahlbeck D, Staskawicz B (2016) CRISPR-Cas9 mediated mutagenesis of a *DMR6* ortholog in tomato confers broad-spectrum disease resistance. bioRxiv:064824

Pavan S, Jacobsen E, Visser RGF, Bai Y (2009) Loss of susceptibility as a novel breeding strategy for durable and broad-spectrum resistance. Molecular Breeding 25:1

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256-261

Piatek A, Ali Z, Baazim H, Li L, Abulfaraj A, Al-Shareef S, Aouida M, Mahfouz MM (2015) RNA-guided transcriptional regulation in planta via synthetic dCas9-based transcription factors. Plant Biotechnol J 13:578-589

Pouchieu C, Galan P, Ducros V, Latino-Martel P, Hercberg S, Touvier M (2014) Plasma carotenoids and retinol and overall and breast cancer risk: a nested case-control study. Nutrition and cancer 66:980-988

Pulkkinen M, Gautam M, Lampi A-M, Ollilainen V, Stoddard F, Sontag-Strohm T, Salovaara H, Piironen V (2015) Determination of vicine and convicine from faba bean with an optimized high-performance liquid chromatographic method. Food Research International 76:168-177

Quadrana L, Almeida J, Asís R, Duffy T, Dominguez PG, Bermúdez L, Conti G, Corrêa da Silva JV, Peralta IE, Colot V, Asurmendi S, Fernie AR, Rossi M, Carrari F (2014) Natural occurring epialleles determine vitamin E accumulation in tomato fruits. Nature communications 5:3027

Ralley L, Schuch W, Fraser PD, Bramley PM (2016) Genetic modification of tomato with the tobacco lycopene β -cyclase gene produces high β -carotene and lycopene fruit. Zeitschrift für Naturforschung C 71:295-301

Ribeiro M, Nunes FM, Rodriguez-Quijano M, Carrillo JM, Branlard G, Igrejas G (2018) Next-generation therapies for celiac disease: The gluten-targeted approaches. *Trends in Food Science & Technology* 75:56-71

Risk JM, Selter LL, Krattinger SG, Viccars LA, Richardson TM, Buesing G, Herren G, Lagudah ES, Keller B (2012) Functional variability of the Lr34 durable resistance gene in transgenic wheat. *Plant Biotechnol J* 10:477-487

Rodríguez-Leal D, Lemmon ZH, Man J, Bartlett ME, Lippman ZB (2017) Engineering Quantitative Trait Variation for Crop Improvement by Genome Editing. *Cell* 171:470-480.e478

Rodríguez-Kessler M, Alpuche-Solís AG, Ruiz OA, Jiménez-Bremont JF (2006) Effect of Salt Stress on the Regulation of Maize (*Zea mays* L.) Genes Involved in Polyamine Biosynthesis. *Plant Growth Regulation* 48:175-185

Ronen G, Carmel-Goren L, Zamir D, Hirschberg J (2000) An alternative pathway to beta -carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. *Proc Natl Acad Sci USA* 97:11102–11107

Ross AB, van der Kamp JW, King R, Lê KA, Mejbourn H, Seal CJ, Thielecke F (2017) Perspective: A Definition for Whole-Grain Food Products-Recommendations from the Healthgrain Forum. *Advances in nutrition (Bethesda, Md)* 8:525-531

Rustgi S, Shewry P, Brouns F, Deleu LJ, Delcour JA (2019) Wheat Seed Proteins: Factors Influencing Their Content, Composition, and Technological Properties, and Strategies to Reduce Adverse Reactions. *Comprehensive reviews in food science and food safety* 18:1751-1769

Salentijn EM, Goryunova SV, Bas N, van der Meer IM, van den Broeck HC, Bastien T, Gilissen LJWJ, Smulders MJM (2009) Tetraploid and hexaploid wheat varieties reveal large differences in expression of alpha-gliadins from homoeologous Gli-2 loci. *BMC Genomics* 10:48-48

Sánchez-León S, Gil-Humanes J, Ozuna CV, Giménez MJ, Sousa C, Voytas DF, Barro F (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnol J* 16:902-910

Sandhu D, Pudussery MV, Kumar R, Pallete A, Markley P, Bridges WC, Sekhon RS (2020) Characterization of natural genetic variation identifies multiple genes involved in salt tolerance in maize. *Functional & Integrative Genomics* 20:261-275

Santosh Kumar VV, Verma RK, Yadav SK, Yadav P, Watts A, Rao MV, Chinnusamy V (2020) CRISPR-Cas9 mediated genome editing of drought and salt tolerance (*OsDST*) gene in indica mega rice cultivar MTU1010. *Physiology and Molecular Biology of Plants* 26:1099-1110

Sasaki A, Ashikari M, Ueguchi-Tanaka M, Itoh H, Nishimura A, Swapan D, Ishiyama K, Saito T, Kobayashi M, Khush GS, Kitano H, Matsuoka M (2002) A mutant gibberellin-synthesis gene in rice. *Nature* 416:701-702

Sashidhar N, Harloff HJ, Potgieter L, Jung C (2020) Gene editing of three BnITPK genes in tetraploid oilseed rape leads to significant reduction of phytic acid in seeds. *Plant Biotechnology Journal* 18:2241-2250

Sathe SK (2012) Chemistry and Implications of Antinutritional Factors in Dry Beans and Pulses. *Dry Beans and Pulses Production, Processing and Nutrition*, pp 359-377

Savary S, Ficke A, Aubertot J-N, Hollier C (2012) Crop losses due to diseases and their implications for global food production losses and food security. *Food Security* 4:519-537

Scheben A, Wolter F, Batley J, Puchta H, Edwards D (2017) Towards CRISPR/Cas crops - bringing together genomics and genome editing. *The New phytologist* 216:682-698

Schnable JC (2015) Genome Evolution in Maize: From Genomes Back to Genes. *Annual Review of Plant Biology* 66:329-343

Seymour DK, Becker C (2017) The causes and consequences of DNA methylome variation in plants. *Curr Opin Plant Biol* 36:56-63

Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, Zhang K, Liu J, Xi JJ, Qiu J-L, Gao C (2013) Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature Biotechnology* 31:686-688

Shelef O, Weisberg PJ, Provenza FD (2017) The Value of Native Plants and Local Production in an Era of Global Agriculture. *Frontiers in plant science* 8:2069-2069

Shen L, Liu Z, Xu Y (2016) Regularized polarimetric target detection in the presence of inhomogeneous and/or non-stationary clutter. 2016 CIE International Conference on Radar (RADAR), pp 1-4

Shi J, Gao H, Wang H, Lafitte HR, Archibald RL, Yang M, Hakimi SM, Mo H, Habben JE (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnol J* 15:207-216

Shi J, Lai J (2015) Patterns of genomic changes with crop domestication and breeding. *Current Opinion in Plant Biology* 24:47-53

Shim YY, Gui B, Arnison PG, Wang Y, Reaney MJT (2014) Flaxseed (*Linum usitatissimum* L.) bioactive compounds and peptide nomenclature: A review. *Trends in Food Science & Technology* 38:5-20

Sima P, Vannucci L, Vetvicka V (2018) β -glucans and cholesterol (Review). *Int J Mol Med* 41:1799-1808

Simmonds, J., Scott, P., Brinton, J., Mestre, T.C., Bush, M., Del Blanco, A., Dubcovsky, J., Uauy, C. (2016) A splice acceptor site mutation in TaGW2-A1 increases thousand grain weight in tetraploid

and hexaploid wheat through wider and longer grains. *Theoretical and Applied Genetics* 129, 1099-112.

Smith BD (2006) Eastern North America as an independent center of plant domestication. *Proceedings of the National Academy of Sciences* 103:12223-12228

Smith BD, Yarnell RA (2009) Initial formation of an indigenous crop complex in eastern North America at 3800 B.P. *Proceedings of the National Academy of Sciences* 106:6561-6566

Sollid LM, Qiao S-W, Anderson RP, Gianfrani C, Koning F (2012) Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. *Immunogenetics* 64:455-460

Song, X.J., Huang, W., Shi, M., Zhu, M.Z., Lin, H.X. (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. *Nature Genetics* 39, 623-30.

Sun L, Yuan B, Zhang M, Wang L, Cui M, Wang Q, Leng P (2012) Fruit-specific RNAi-mediated suppression of *SINCE1* increases both lycopene and β -carotene contents in tomato fruit. *Journal of experimental botany* 63:3097-3108

Sundaramoorthy, J., Park, G.T., Komagamine, K., Tsukamoto, C., Chang, J.H., Lee, J.D., Kim, J.H., Seo, H.S., and Song, J.T. (2019). Biosynthesis of DDMP saponins in soybean is regulated by a distinct UDP-glycosyltransferase. *New Phytologist* 222, 261-274.

Swinnen G, Goossens A, Pauwels L (2016) Lessons from Domestication: Targeting Cis-Regulatory Elements for Crop Improvement. *Trends in plant science* 21:506-515

Tan SH, Mailer RJ, Blanchard CL, Agboola SO (2011) Canola proteins for human consumption: extraction, profile, and functional properties. *J Food Sci* 76:R16-R28

Taylor JE, Hatcher PE, Paul ND (2004) Crosstalk between plant responses to pathogens and herbivores: a view from the outside in. *Journal of Experimental Botany* 55:159-168

Thambugala D, Pozniak CJ, Kumar S, Burt AJ, Wise IL, Smith MAH, Fox SL, Costamagna AC, McCartney CA. Genetic analysis of oviposition deterrence to orange wheat blossom midge in spring wheat. *Theor Appl Genet.* 2021 Feb;134(2):647-660. doi: 10.1007/s00122-020-03720-y. Epub 2020 Nov 16. PMID: 33200319.

Thomas, J. & Fineberg, N. & Penner, G. & McCartney, Curt & Aung, Thantoe & Wise, I. & McCallum, Brent. (2005). Chromosome location and markers of *Sm1*: A gene of wheat that conditions antibiotic resistance to orange wheat blossom midge. *Molecular Breeding* 15: 183-192. 10.1007/s11032-004-5041-2.

Ton LB, Neik TX, Batley J (2020) The Use of Genetic and Gene Technologies in Shaping Modern Rapeseed Cultivars (*Brassica napus* L.). *Genes* 11:1161

Tuncel A, Corbin KR, Ahn-Jarvis J, Harris S, Hawkins E, Smedley MA, Harwood W, Warren FJ, Patron NJ, Smith AM (2019) Cas9-mediated mutagenesis of potato starch-branching enzymes generates a range of tuber starch phenotypes. *Plant Biotechnology Journal* 17:2259-2271

Tye-Din JA, Stewart JA, Dromey JA, Beissbarth T, van Heel DA, Tatham A, Henderson K, Mannering SI, Gianfrani C, Jewell DP, Hill AV, McCluskey J, Rossjohn J, Anderson RP (2010) Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. *Science translational medicine* 2:41ra51

Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., Dubcovsky, J. (2006) A NAC gene regulating senescence improves grain protein, zinc and iron content in wheat. *Science*. 314, 1298–1300.

Ujjal Kumar N, It, sup, gt, It, sup, gt, Hoy-Taek K, It, sup, gt, It, sup, gt, Khadiza K, It, sup, gt, It, sup, gt, Jong-In P, It, sup, gt, It, sup, gt, Kwon-Kyoo K, It, sup, gt, It, sup, gt, and Ill-Sup N, It, sup, gt, It, sup, gt (2016) Modification of Fatty Acid Profiles of Rapeseed (<i>Brassica napus</i> L.) Oil for Using as Food, Industrial Feed-Stock and Biodiesel. *Plant Breed Biotech* 4:123-134

van Schie CCN, Takken FLW (2014) Susceptibility Genes 101: How to Be a Good Host. *Annual Review of Phytopathology* 52:551-581

Varotto S, Tani E, Abraham E, Krugman T, Kapazoglou A, Melzer R, Radanović A, Miladinović D (2020) Epigenetics: possible applications in climate-smart crop breeding. *Journal of Experimental Botany* 71:5223-5236

Veillet F, Chauvin L, Kermarrec MP, Sevestre F, Merrer M, Terret Z, Szydłowski N, Devaux P, Gallois JL, Chauvin JE (2019) The *Solanum tuberosum* GBSSI gene: a target for assessing gene and base editing in tetraploid potato. *Plant cell reports* 38:1065-1080

Walkowiak, S., Gao, L., Monat, C. *et al.* Multiple wheat genomes reveal global variation in modern breeding. *Nature* **588**, 277–283 (2020)

Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, Liu Y-G, Zhao K (2016a) Enhanced Rice Blast Resistance by CRISPR/Cas9-Targeted Mutagenesis of the ERF Transcription Factor Gene OsERF922. *PLoS one* 11:e0154027

Wang F, Xu Y, Li W, Chen Z, Wang J, Fan F, Tao Y, Jiang Y, Zhu Q-H, Yang J (2020) Creating a novel herbicide-tolerance OsALS allele using CRISPR/Cas9-mediated gene editing. *The Crop Journal*

Wang W, Qin Q, Sun F, Wang Y, Xu D, Li Z, Fu B (2016b) Genome-Wide Differences in DNA Methylation Changes in Two Contrasting Rice Genotypes in Response to Drought Conditions. *Frontiers in plant science* 7:1675-1675

Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu J-L (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nature Biotechnology* 32:947-951

Wang, W., Simmonds, J., Pan, Q., Davidson, D., He, F., Battal, A., Akhunova, A., Trick, H.N., Uauy, C., Akhunov, E. (2018) Gene editing and mutagenesis reveal inter-cultivar differences and additivity in the contribution of TaGW2 homoeologues to grain size and weight in wheat. *Theoretical and Applied Genetics* 131, 2463–2475.

Wang Z, Ma L-Y, Cao J, Li Y-L, Ding L-N, Zhu K-M, Yang Y-H, Tan X-L (2019) Recent Advances in Mechanisms of Plant Defense to *Sclerotinia sclerotiorum*. *Frontiers in Plant Science* 10

Wei D, Cui Y, He Y, Xiong Q, Qian L, Tong C, Lu G, Ding Y, Li J, Jung C, Qian W (2017) A genome-wide survey with different rapeseed ecotypes uncovers footprints of domestication and breeding. *Journal of Experimental Botany* 68:4791-4801

Wickramasuriya SS, Yi Y-J, Yoo J, Kang NK, Heo JM (2015) A review of canola meal as an alternative feed ingredient for ducks. *J Anim Sci Technol* 57:29-29

Xu R, Li H, Qin R, Wang L, Li L, Wei P, Yang J (2014) Gene targeting using the *Agrobacterium tumefaciens*-mediated CRISPR-Cas system in rice. *Rice* 7:5

Yaish MW, Al-Lawati A, Al-Harrasi I, Patankar HV (2018) Genome-wide DNA Methylation analysis in response to salinity in the model plant caliph medic (*Medicago truncatula*). *BMC Genomics* 19:78

Yang Y, Zhu G, Li R, Yan S, Fu D, Zhu B, Tian H, Luo Y, Zhu H (2017) The RNA Editing Factor SIORRM4 Is Required for Normal Fruit Ripening in Tomato. *Plant physiology* 175:1690-1702

Zafar, S.A., Zaidi, S.S.-e.-A., Gaba, Y., Singla-Pareek, S.L., Dhankher, O.P., Li, X., Mansoor, S., and Pareek, A. (2019). Engineering abiotic stress tolerance via CRISPR/ Cas-mediated genome editing. *Journal of Experimental Botany* 71, 470-479.

Zaidi SS, Mukhtar MS, Mansoor S (2018) Genome Editing: Targeting Susceptibility Genes for Plant Disease Resistance. *Trends in biotechnology* 36:898-906

Zhang A, Liu Y, Wang F, Li T, Chen Z, Kong D, Bi J, Zhang F, Luo X, Wang J, Tang J, Yu X, Liu G, Luo L (2019) Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Molecular Breeding* 39:47

Zhang J, Zhang X, Chen R, Yang L, Fan K, Liu Y, Wang G, Ren Z, Liu Y (2020) Generation of Transgene-Free Semidwarf Maize Plants by Gene Editing of Gibberellin-Oxidase20-3 Using CRISPR/Cas9. *Frontiers in Plant Science* 11

Zhang K, Raboanatahiry N, Zhu B, Li M (2017a) Progress in Genome Editing Technology and Its Application in Plants. *Front Plant Sci* 8:177

Zhang M, Cao Y, Wang Z, Wang ZQ, Shi J, Liang X, Song W, Chen Q, Lai J, Jiang C (2018a) A retrotransposon in an HKT1 family sodium transporter causes variation of leaf Na(+) exclusion and salt tolerance in maize. *The New phytologist* 217:1161-1176

Zhang Y, Bai Y, Wu G, Zou S, Chen Y, Gao C, Tang D (2017b) Simultaneous modification of three homoeologs of TaEDR1 by genome editing enhances powdery mildew resistance in wheat. *The Plant Journal* 91:714-724

Zhang Y, Massel K, Godwin ID, Gao C (2018b) Applications and potential of genome editing in crop improvement. *Genome Biology* 19:210

Zhang, Y., Li, D., Zhang, D., et al. (2018) Analysis of the functions of TaGW2 homoeologs in wheat grain weight and protein content traits. *Plant Journal* 94, 857–866.

Zhou L, Tian S, Qin G (2019) RNA methylomes reveal the m⁶A-mediated regulation of DNA demethylase gene SIDML2 in tomato fruit ripening. *Genome biology*, p 156

Zhou Y, Massonnet M, Sanjak JS, Cantu D, Gaut BS (2017) Evolutionary genomics of grape (*Vitis vinifera* ssp. *vinifera*) domestication. *Proceedings of the National Academy of Sciences* 114:11715-11720

Zhu J-K (2016) Abiotic Stress Signaling and Responses in Plants. *Cell* 167:313-324

Zong G, Gao A, Hu FB, Sun Q (2016) Whole Grain Intake and Mortality From All Causes, Cardiovascular Disease, and Cancer: A Meta-Analysis of Prospective Cohort Studies. *Circulation* 133:2370-2380

Zsögön A, Čermák T, Naves ER, Notini MM, Edel KH, Weini S, Freschi L, Voytas DF, Kudla J, Peres LEP (2018) De novo domestication of wild tomato using genome editing. *Nature Biotechnology* 36:1211-1216

Zuo W, Chao Q, Zhang N, Ye J, Tan G, Li B, Xing Y, Zhang B, Liu H, Fengler KA, Zhao J, Zhao X, Chen Y, Lai J, Yan J, Xu M (2015) A maize wall-associated kinase confers quantitative resistance to head smut. *Nature Genetics* 47:151-157